Is *Fusarium torulosum* the causal agent of kikuyu poisoning in Australia?

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Abstract. *Fusarium torulosum* was isolated consistently from the leaves of kikuyu plants collected from a pasture near Maitland, NSW, on which grazing cattle had been affected by kikuyu poisoning. This fungus is known to produce the toxins wortmannin and butenolide, both of which can produce clinical signs and pathological changes similar to those exhibited by cattle with kikuyu poisoning. The available evidence suggests that *F. torulosum* might be the casual agent of kikuyu poisoning in Australia.

Poisoning of cattle, sheep and goats after grazing on kikuyu (*Pennisetum clandestinum*) pastures has been reported occasionally since the early 1960s from New Zealand, Kenya, Rhodesia, South Africa and Australia (Bourke 2007). In Australia, kikuyu poisoning has occurred in Queensland (Wells 1958), New South Wales (NSW) (Wong *et al.* 1987) and Western Australia (Gabbedy *et al.* 1974). Kikuyu poisoning causes drooling of saliva, dehydration, abdominal pain, sham drinking, depression, incoordination and recumbency (Bourke 2007). Mortality rates of 8.9–32% have been reported for cattle, with 16.7–95.6% of affected animals dying. In Australia, kikuyu poisoning occurs in autumn after a long period of summer drought, when rapid grass growth is initiated by rainfall or irrigation. Outbreaks tend to be geographically restricted, sudden in onset, short in duration, and sporadic, making identification of the causal agent difficult. Bourke (2007) reviewed, in detail, the potential agents which have been implicated in the past. He dismissed nitrogen-based causes such as nitrate–nitrite poisoning or peracute ammonia toxicity because there was no clinical, pathological or biochemical evidence. An association between armyworm caterpillars (species of *Mythimna*, *Persectania*, *Pseudaletia* and *Spodoptera*) and kikuyu poisoning has been reported by several authors (Busch *et al.* 1969; Cordes *et al.* 1969; Smith and Martinovich 1973), but Bourke (2007) considers that the association is more likely to be casual, rather than armyworms being directly responsible.

There are conflicting reports of the association of fungi with toxic kikuyu pastures. Although cultures of species of the common pasture fungi *Myrothecium* and *Phoma* were reported to cause kikuyu poisoning-like symptoms in ruminants (Di Menna and Mortimer 1971; Martinovich and Smith 1972), no consistent relationship between these fungi and kikuyu poisoning has been established (Bourke 2007). In a study of fungi isolated from five

toxic kikuyu pastures in NSW Wong *et al.* (1987) found that *Fusarium semitectum*, *Fusarium moniliforme* var. *subglutinans* (syn. *Fusarium subglutinans*), *Penicillium* spp., and *Phoma* sp. were the most common. Only the first fungus was consistently isolated from all five pastures, but it was also isolated from two adjacent kikuyu paddocks in which poisoning had not occurred.

In late March 2006, samples of kikuyu grass from a pasture at Seaham (near Maitland), NSW, in which three of 15 cows had died after displaying symptoms of kikuyu poisoning were forwarded to one of us (MJR) by overnight courier. Short sections ($n = 20$; ~10 mm long) of the sheaths and blades of leaves were excised and placed in 10 mL of 95% ethanol:lactic acid (1:1) in a 20 mL McCartney bottles, which were then partly immersed in a 60°C water bath overnight to remove chlorophyll. The clearing agent was replaced with a staining solution of lactophenol–trypan blue (0.1%) which was then heated at 60°C for 3 h. The staining solution was poured off, and replaced with lactic acid, which was heated to 60°C for 20 min. The leaf pieces were removed, placed on microscope slide, covered with a glass coverslip and examined at $\times 200$ under a compound microscope. Septate hyphae, 2–4 μm in diameter, were observed between the parenchyma cells in both the leaf sheaths and leaf blades.

Ten sections each of leaf sheaths and blades were surface-sterilised in an aqueous solution of sodium hypochlorite (1% available chlorine) for 3 min, rinsed twice in sterile distilled water, then placed on the surfaces of 1.5% water agar in 9 cm diameter Petri dishes. The plates were incubated in filtered sunlight at 22–25°C, and after 5 days a fungus with identical colony characteristics had grown from >75% of all plated sections. Hyphal tips (~20 μm long) were excised from a representative sample of the colonies and placed on the surfaces of potato dextrose agar (PDA) (Oxoid, Basingstoke, England)

on 9 cm diameter Petri dishes, which were incubated in the dark at 25°C. After 14 days, the colonies which developed from the hyphal tips were composed of dense white-light orange mycelium with a bright white, lobed margin. The reverse of the colonies were red to red-brown. In colonies grown on half strength PDA in the dark at 25°C, falcate, 4–5-septate macroconidia, and aseptate or 1-septate microconidia were common, and chlamydospores developed in chains. An isolate was forwarded to the Plant Disease Diagnostic Unit at the Botanic Gardens Trust, Sydney, where it was identified as *Fusarium torulosum* (ref. I06/276) using the morphological characteristics of propagules on carnation leaf agar, and the culture characteristics on PDA, as outlined in Leslie and Summerell (2006).

Our findings indicate that *F. torulosum* is endophytic in the leaves of *P. clandestinum* plants from a pasture displaying kikuyu poisoning. Although there is no direct link between *F. torulosum* and kikuyu poisoning, circumstantial evidence suggests that the link may exist. *Fusarium torulosum* has been isolated from soil in pastures and under pines in Australia (Benyon *et al.* 2000), from wheat kernels in Nepal (Desjardins *et al.* 2000), and from beet, potato and wheat in Europe (Desjardins 2006). *Fusarium torulosum* is known to produce the toxins wortmannin (Thrane and Hansen 1995), which has a very low LD₅₀ in rats (4 mg/kg bodyweight) (Abbas and Mirocha 1988), butenolide, which is lethal in cattle at 40 mg/kg bodyweight (Tookey and Grove 1972), enniatins and moniliformin (Desjardins 2006). Wortmannin causes potentially lethal acute inflammation in the stomach, intestines, heart and thymus in mice and rats (Abbas and Mirocha 1988; Gunther *et al.* 1989), while butenolide causes potentially lethal acute inflammation in the forestomach in cattle (Tookey and Grove 1972). These effects are consistent with changes found in cattle affected by kikuyu poisoning (Bourke 2007).

In a study of *Fusarium* species isolated from soil from New Zealand pastures, Abbas *et al.* (1991) reported that a culture of *Fusarium sambucinum* produced a high level (40 mg/kg) of wortmannin, while Bosch *et al.* (1989) reported even higher levels (up to 272 mg/kg) of the compound in cultures of the same species from New Zealand pastures. Nirenberg (1995) differentiated three species within *F. sambucinum sensu lato*, *F. sambucinum sensu stricto*, *Fusarium venenatum*, and *F. torulosum*. Desjardins (2006) considers that ‘the species (*F. torulosum*) perhaps continues to be misidentified as *F. sambucinum*’. It is highly likely that the isolates of *F. sambucinum* in the New Zealand studies were *F. torulosum*, because it is the only *Fusarium* species known to produce wortmannin (Desjardins 2006). Many isolates collected in Australia and New Zealand, and identified as *F. sambucinum sensu stricto*, have since been shown to be *F. torulosum* (B. A. Summerell, unpubl. data).

Consequently, it seems likely that *F. torulosum* is a common inhabitant of soil and plants in pastures, and that at least some isolates produce wortmannin and butenolide, toxic agents which can cause effects that are very similar to those produced in cattle grazing on toxic kikuyu pastures. Further research is needed to conclusively prove or disprove the hypothesis that either wortmannin or butenolide produced by *F. torulosum*

is the cause of kikuyu poisoning in Australia. Samples from toxic kikuyu pastures need to be assayed for wortmannin and butenolide, isolates of *F. torulosum* collected from these pastures must be assessed for their ability to produce wortmannin or butenolide *in vitro*, and feeding studies to quantify the clinical effects of wortmannin and butenolide on cattle should be undertaken.

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